

# CALIFORNIA PITCH CANKER TASK FORCE

February 24, 2005 Meeting

## MINUTES

The meeting was called to order at 9:15 a.m. by Chairman Steve Staub.

### APPROVAL OF MINUTES

**Wally Mark moved that the minutes be approved as read. Susan Frankel seconded the motion, which was passed unanimously.**

Chairman Staub turned the meeting over to Dr. Tom Gordon, of the University of California at Davis.

### RESEARCH PRESENTATIONS

Dr. Gordon thanked the supporters of the work being done at UC Davis, including the Pebble Beach Company, the U.S. Forest Service, the Del Monte Forest Foundation, California Department of Fire and Forestry, the California Christmas Tree Growers, the USDA Exotic Pest Disease Program. He encouraged members of the Task Force and other visitors to ask questions of the presenters today. Dr. Gordon then introduced the first speaker

**Inheritance of Virulence in Fungal Hybrids from the *Gibberella fujikouri* Species Complex** by Chris Friel

Mr. Friel began by explaining that this is a complex group of fungi. Each species is made distinct because they are reproductively isolated. It was believed that these fungi were asexual, but it was found that they were sexual in the laboratory. They are commonly known as fusarium. *Fusarium circinatum* is in this group and is known as *Gibberella circinatum*.

The requirements for a successful cross are:

1. Compatibility at the MAT locus, which is determined by PCR (the mating type)
2. One isolate must be a female fertile, cultured on carrot agar

*Gibberella circinata* is *fusarium circinatum* in the sexual state. It is known as Species H MAT 1, Isolate: GL34. H refers to the mating population. It is mated with *Gibberella subglutinans*, which is *Fusarium subglutinans*, Species E MAT2, Isolate: Fst 59. These are collected from seeds in Mexico, which are mated randomly from other isolates in Mexico.

For female fertility, they grow the fungi on carrot agar. The others are grown on slants. They are then put in the growth chamber. If successful, they grow mating structures. The progeny are collected and are evaluated to see if they really are the sexual product of the virulent product and the avirulent product (*subglutinans*). The IMPACT trees are then inoculated and the lesions are evaluated. In this project, 277 progeny were collected under the microscope and 180 were tested and evaluated by looking at genetic markers that were unique to each parent. Approximately 51.8% were MAT 1, which indicated nearly a 50-50 distribution of mating type. When the DNA segment was checked to determine what population type the progeny actually were from, it was determined that most of the F1 progeny were from the two original parents, with 2/3 from the avirulent parent.

Pathogenicity is also a heritable trait from a sexual cross. When tested, 176 of the 180 were avirulent. Therefore, with an even distribution of the mating type, there was a greater representation of the avirulent type. There were 4 progeny that were virulent; two of them were clearly recombinant types.

Mr. Friel stated that in their follow up they repeated the process on the 4 progeny that were virulent and on a few others. The virulent progeny are still virulent and the avirulent progeny were still under the threshold of virulence.

Dr. Volger asked if Mr. Friel did fitness tests to determine if the progeny could survive out of the lab. Mr. Friel responded that they did not do fitness tests out of the lab; that all the work was done in the lab. He added that it was difficult to recreate the cross, but that recently they were able to repeat the cross a second time.

Dr. Volger asked about the significance of the fact that they are biologically isolated. Mr. Friel stated that it is a hybrid progeny which probably could not reproduce. This leads credibility to the belief that they are of a common origin and are related.

Dr. Frankel asked if the correct name is *Gibberella circinata*. Mr. Friel stated that yes that is the correct name, but that no one would be able to determine what you are referring to with the name. That is the problem with asexual names for fungal pathogens. It adds to the confusion.

Dr. Wood asked if they have found mating types in nature. Mr. Friel noted that they had found mating types in nature but that they don't mate. He stated that there is no genetic evidence that there has been sexual reproduction in the population in California. He added that perhaps in the Southeast there is sexual reproduction because there is more vegetation and an older population.

Dr. Volger asked if there are only asexual isolates able to survive in the outside world. Mr. Friel noted that they cannot take these progeny outside, but they would probably survive if they were outside because they make lesions. However, it is not likely that they would establish a new strain through vegetative hybridization.

### **The Effect of Temperature on Mycelial Growth, Spore Germination and Wound Healing in *Fusarium circinatum*** by Annie Inman

Ms Inman began by describing that the distribution of pitch canker in California is in the coastal region and in mild climates. Pitch canker infections are not found in the colder mountain climates in California.

Isolates across a wide geographic range were screened for variance with respect to the effect of temperature on mycelial growth. Determining the radial growth measured across all isolates showed that temperature has a significant effect on the radial growth rate of the isolates. There is more growth at high temperatures. It was determined that different isolates appear to be more cold tolerant than other isolates. For example A3Mex, FL10, Fsp254 and Fsp456 appear to be more cold tolerant relative to other isolates.

The effects of temperature on spore germination were examined for isolates of diverse geographic origin. In germination across all isolates it was determined that the percent germination was not effected by temperatures between 10C and 25C for most isolates but that the time for germination varies greatly between temperatures. Studies found that A3Mex and Fsp63 have unique germination patterns and were

affected by temperatures. Additionally, at 5C, conidia germinate after about 60 days. At 5C, Fsp456 appears to germinate before other isolates.

The study concluded that temperature affects the rate of both mycelial growth and spore germination. Temperature does not affect percent germination. A study regarding the affects of temperature on progeny is in progress. Preliminary data suggest that cold temperature limits the ability of the pathogen to infect.

Some questions that need to be answered are how does the temperature and slowness of germination affect the wound of trees? How long to wounds remain susceptible to infection? Trees were wounded all at the same time and were inoculated on different days. The inoculations were replicated in winter, spring and fall. Preliminary results suggest low winter infection, but the hypothesis is that the spring and fall ones will be very successful.

Mr. Hawley asked about the California isolate, inquiring what stand that was from. Dr. Gordon replied that it could have come from Badger Hill. Nadir Erilgin asked if, in cold temperature, the isolates die. He was told that they would eventually germinate, but it just takes more time.

Dr. Volger asked if they were going to address the extremely hot temperatures, greater than 25C. Ms Inman stated that they were more worried about the ability of the disease to spread to places where it is colder, so that is what the research is investigating at the moment. Dr. Gordon stated that they have other studies regarding the effect of them drying out in the hot weather. Dr. Volger stated that a general warming trend is being observed and it will be interesting to see what that will do. Dr. Mark asked if there were any tests with variable temperatures to simulate the diurnal temperatures of nature. Ms Inman stated that there are some outdoor experiments that are currently being conducted.

Chairman Staub asked about wound susceptibility and about pitch as a growth medium being a significant inhibitor. He asked if that was being examined here or is it too small for that. Ms Inman stated that she was not sure if there will be an actual analysis, but that she is taking note of whether or not the pitch was on the wound and if it didn't make it less likely to form a lesion. Mr. Friel stated that those experiments were done in incredibly saturated atmospheres, so no tree in nature could inhibit the growth of pitch canker.

#### **Update on Study of Seedling Infections Initiated in 2004** by Dr. Brenna Aegar

Dr. Aegar stated that they study was to determine the mortality rate of seedlings emerging from infested soil and how prior fumigation of the soil affects the response of emerging seedlings to soil inoculum.

The study used natural soil treated with Basamid and then artificially infested with spores at concentrations of 100, 1,000, 10,000 and 100,000 spores per gram of soil. Each soil in the greenhouse was planted with 240 seeds with 4 replicates. The dying seedlings were plated on selected medium and scored for *Fusarium circinatum*.

There was a dramatic response to the ten-fold increase in inoculum, with up to 100 percent mortality in high levels of concentration. This was in a fairly short time frame, so it could have come up slightly over time if they stayed in the soil.

When looking at the effect of fumigation prior to spores being placed in the soil, at the point where the soil has not been fumigated, the seedling mortality is slightly lower because there are microorganisms in

the soil that would be antagonistic and would reduce the capability to infect. In the fumigated soil there is a microbiological vacuum.

Mr. Hawley noted that in the Woody Guidelines the recommendation is to chip and broadcast chips back on the property. The inoculum level would go up in native stands based on that. He noted that perhaps broadcasting the chips back on properties would reduce regeneration significantly. Dr. Gordon stated that it is still a good idea and he wouldn't change the recommendation. Dr. Aegar added that the mulching effect would help. Dr. Gordon stated that there is significant attrition over time. Also, there is emergence through needles. He stated that the viability of the spores in the duff declines over time. The magnitude of the contribution by spreading the chips would be relative minor. Dr. Wood stated that it would also reduce the insect population by about 95%. Dr. Aegar added that there are many other things that would affect the regeneration.

### **Quantifying the Risk of Pitch Canker to Susceptible Pines in California** by Dr. Brenna Aegar

Dr. Aegar stated that the objectives of this study were to characterize the conditions of temperature and moisture required for infection by *Fusarium circinatum*, to develop a mathematical model to predict infection frequency based on temperature and relative humidity and to validate the model under field conditions.

Observational plots were developed in the Monterey Peninsula and San Francisco Bay area. The study was to monitor changes in the disease from year to year in 10 plots, checking them every six months. The environmental conditions were monitored with "mini" weather stations which measured temperature and humidity. In the observational plots, the numbers of tips on a set of 20 trees from different areas were determined. In six months, the numbers of new tips were counted. Then the study attempted to correlate the number of new infections with the weather during that six month period.

The same study conducted growth chamber experiments, where the temperature was controlled to determine how the temperature and duration of wetness affected infection of wounds. They made artificial wounds and introduced the pathogen. There is no data from these experiments because it hasn't turned out as expected; they found very high infections rates. This might have occurred because they moved the specimens from the cold chamber to the fluctuating temperature chamber.

In the experimental field plots, they were using a number of different areas throughout the year, so there were different conditions of humidity and temperature. They sprayed them with spore suspension and allowed it to dry, after which they wounded the branch. In the initial experiments, one-half of the branches were bagged overnight. In subsequent experiments, other treatments were explored. The weather conditions at the field plots were monitored.

It was found that in the nine initial experiments, there was no effect of bagging. Even in the number of different temperature and humidity conditions, the bagging didn't significantly affect the infection rate. While monitoring the weather at the plots, without knowing how long wounds become susceptible, they didn't know how many hours or what type of weather to look for.

The study wanted to determine if wound depth would affect infection. They wanted to determine if there was an interaction of wound depth and environmental parameters, if the drill bit made the wound so deep that that bagging would affect it. This deep drill is not representative of a beetle wound, which is much closer to a dissecting needle. Chris Friel handcrafted a wounding implement by which a dissecting needle could be extended beyond a plastic pipette and make consistent wounds over several days. After comparing the deep and shallow wounds, they found still slightly higher infection rates in the deeper

wounds and so determined that there is an interaction between the depth of the wounds and the environmental parameters.

They next added water into wounds after making them. In all the studies, the infection rate was higher in the wounds in which water was added.

Dr. Aegar stated that they did establish a correlation between weather and risk of infection. They were attempting to get an idea of how temperature and relative humidity might be a limiting factor of pitch canker and a factor in the risk of pitch canker spreading in California. Dr. Aegar presented a slide which indicated the areas in California that have do not have pitch canker limiting factors and also areas where sometimes during the year the weather would be conducive to the spread of pitch canker.

Chairman Staub asked why they thought that the wound susceptibility only extends as long as 96 hours and why not a month or two? Dr. Aegar stated that different types of wounds take longer to heal. She added that they are mostly looking at the wounds made by insects and she wouldn't know the effect on larger wounds.

Mr. Hawley asked about the take-home message for land managers? He stated that there are a lot of problems with arborists and county tree workers and independent tree workers being hired by people doing lot clearing and they wound trees, both superficially and deeply. He added that even trimming trees for line clearances causes wounding. Dr. Gordon answered that they know pruning wounds can become infected, but that they tend to dry out and become unsuitable for infection sites. He said that is why that is not where an important infection gets started. Dr. Aegar added that it best to clean the pruning instruments according to the standards that have already been set.

### **Provenance Seedlings – Do Different Populations Exhibit Variable Susceptibility to Pitch Canker?**

by Sharon Kirkpatrick (founding member of the Pitch Canker Task Force)

Dr. Kirkpatrick stated that they have found that conifers in California differ in susceptibility to *Fusarium circinatum*. This is true for both seeds and seedlings from both a single tree and a population. The study is to determine if there is a differential response to the pathogen based on population location (provenance).

When a Gray pine is inoculated and develops a pitch canker lesion, it has been determined that the longer the lesion, the higher the level of susceptibility. There were four population locations for Gray pine, Lake County, Butte County, Santa Barbara County and Contra Costa County. Gray pines proved to be susceptible in all provenances.

Sugar pine has a lot of resin and a different resin component structure. There are 8 provenances of sugar pine: San Bernardino County, Siskiyou County, Shasta County, Mendocino County, East Tahoe County, Lake County, Tulare County and Tuolumne County. The first set of trees had no lesions, but the second and thirds sets of trees had very large lesions. It was determined that from San Bernardino County to Tuolumne County there are some differences in levels of susceptibility.

Dr. Kirkpatrick made the observation that Torrey pine seedlings were often indistinct and the seedling lesion margins appeared water soaked with darker green around the edges. At first this was ignored, so the lesions were considered small on Torrey pines, but she determined that they may be longer than she first thought. She noted after the first set of Gray and Sugar pine seedlings that there was a similar phenomenon, while control Monterey pine seedlings developed typical discolored lesions.

The resulting solution was to rate the seedling's lesions after 4 weeks instead of 3 weeks. This is equivalent to Monterey pine lesions at 3 weeks based on girdling response to lesion length. The lesions for Gray and Sugar pines were then quite distinct and easy to measure. Species vary in response time, which gives a better reading of their susceptibility. They will be repeating inoculations on additional sets of seedlings.

Mr. Hawley asked if the Gray pine could be transporting less water than then Monterey pine because it hardly has any foliage, so the lesions might take longer to grow because they are not being fed. Dr. Kirkpatrick noted that Sugar pines are the opposite.

Dr. Wood asked if they had a natural isolate from a Gray pine. Dr. Kirkpatrick stated that they did not have a natural isolate from a Gray pine or a Torrey pine.

### **Pebble Beach Seedlings – Inheritance of Resistance** by Sharon Kirkpatrick

The object of this study is to determine if parent Monterey pines pass resistance on to their progeny and if there is a correlation between parent tree and half-sibling progeny.

Parent trees were inoculated with *Fusarium circinatum* and their average lesion length was determined. There were 8 cone-bearing trees included in the trial and a total of 1,046 seedlings tested for resistance.

Dr. Kirkpatrick noted that parent tree, #28, had an average lesion length of 3.5 mm. That tree produced 197 seedlings with an average lesion length of 24.5 mm.

Dr. Kirkpatrick concluded that seedlings did not appear to inherit resistance from the cone bearer parent. She concluded that there was no evidence to support a benefit of collecting cones from resistant parent trees. She added that there appeared to be no justification for collecting cones from trees slated for removal, as the genes of trees identified as resistant will not differentially enrich the population for resistant genes.

The study attempted to determine if there is a correlation between seedling and sapling response to the pathogen. As trees grow, do they behave the same as they did as seedlings? They reinoculated multiple branches after they turned into saplings and determined that there is some correlation between how the saplings react. While the seedlings only had one lesion measured because they are too small to have more than one, a sapling can be inoculated on 5 or 10 branches, which will show a variability and an average can be obtained.

She concluded that there is a weak but significant correlation between lesion lengths of seedlings and saplings. The seedling lesions are highly variable because there is only one lesion per seedling which limits an estimate of susceptibility. Screening programs using seedlings may misidentify susceptible seedlings as resistant, but this can be a suitable method for large outplantings, where all trees are not expected to survive.

Dr. Wood asked if there was a chance that the second inoculation lesions would be smaller because they were using the same one twice, but Dr. Kirkpatrick said that it was inoculated two years later.

Bill Werner stated that he created 10 clones from resistant parent trees, and there were approximately 20% shorter lesions on the clones than on the parents six years later. He added that he just did a small number of clones, but that they maintained their resistance, that it is genetic makeup and not something that is specific to the site.

## **Susceptibility of Douglas fir to Pitch Canker** by Dr. Thomas Gordon

Dr. Gordon stated that the study was to determine the susceptibility of Douglas fir to resident California strains of pitch canker. He stated that analysis shows that there is no significant difference between the strain of pitch canker in their ability to cause lesions on Douglas fir or Monterey pine. The study also showed that while not all Douglas fir are susceptible to pitch canker, some Douglas fir are clearly susceptible to California strains of pitch canker.

The study also tested Douglas fir against exotic isolates of *Fusarium circinatum* and against progeny of a cross of California strains, which showed no differential ability to cause the disease in Douglas fir. However, the trees themselves did show a variation averaged over the strains with which they were infected.

The study concluded that there is no evidence of significant variation in virulence to Douglas fir among resident California strains of *Fusarium circinatum*, selected exotic strains of *Fusarium circinatum* and progeny of a cross between California strains. Douglas fir is generally at the low end of susceptibility to pitch canker.

Chairman Staub stated that this would make it a perfect candidate for a silent carrier. Dr. Gordon stated that this was a good point. He added that it can be assumed that there are many infected Douglas firs that are just not detected, which makes them an ideal carrier. One of the questions that they are interested in now is how the fungus sporulates on Douglas firs because of the New Zealand infection. Mr. Hawley noted that this opens up a question of critical pathways and Dr. Gordon agreed with that statement.

Dr. Wood noted that there have been symptomatic trees at Año Nuevo that are associated with an insect. He added that there are symptomatic tips in one location and two lesions on the main stem of a Douglas fir, so it isn't only an asymptomatic carrier.

## **Vegetative Compatibility in *Fusarium circinatum*** by Jeness Peterson

The requirements for *Fusarium circinatum* are carbon, water and mineral nutrients. It also requires nitrogen to make amino acids, DNA and proteins. Nit mutants occur and they are unable to use nitrates as a source of nitrogen, which results in very sparse, flat growth on a medium that contains nitrate as the only source of nitrogen. There are two types of nit mutants: 1) Nit 1 has a dysfunctional enzyme; 2) Nit m is missing a cofactor.

To be vegetatively compatible is a form of self-recognition which allows hyphal fusion to occur and can also be used as a marker. In a compatible reaction, Nit m provides a functional enzyme and Nit 1 provides the cofactor and in the middle of the medium where there is cooperation, there is a wild fluffy growth.

New VCG's are generated by sexual recombination, but there is no evidence that there is mating in the wild in California. They also occur in somatic mutation, the mutations which occur as the fungus grows.

In this experiment, 100 spores of Nit m and 100,000 spores of Nit 1 were placed together. These isolates are not vegetatively compatible. The spores germinated, hyphae grew past each other and there was no hyphal fusion. However, if one happened to convert to match, there would be a fluffy spot on the medium. Once you have the fluffy spot it shows they are cooperating. Then they are separated to see if it changed into another VCG.

Mr. Hawley asked if you eliminated the nitrate from the soil in a greenhouse in which you are growing seedlings, could you breed out pitch canker by neutralizing it. Dr. Gordon stated that the wild type of fungus can use a diversity of nitrogen sources, so this isn't a management tool that would have a significant effect on it. If the nit mutants are put out in a natural environment, *Fusarium* would do fine because there are a lot of other nitrogen sources in nature.

Dr. Frankel asked why it was not compatible with itself anymore in the middle plate on the last slide of the presentation and she was told that perhaps the gene wasn't long enough, so it lost something. Dr. Wood asked how the isolates were picked. Other combinations were tried that did not work as well. These isolates were similar to other markers from another experiment, so Dr. Gordon thought it would be a good place to start. He added that this is probably where C2 and C4 came from in California. He stated that he hadn't seen it but it is the principle by which it could happen. Mr. Hawley asked what the odds are of it happening and Dr. Gordon stated that it is a few in a million.

### **Effects of Mechanical Site Preparation on Monterey Pine Regeneration** by David Wood

Dr. Wood stated that in April of 2000 circular plots with a 6-10 meter radius were created. There were 3 plots in each of 6 areas. Each plot was divided into 3 sectors: 1) no treatment (control); 2) mulched; and 3) all vegetation removed. All seedlings were removed from the control areas. Measurements were taken of the percent plant cover by each species categories and the abundance and percentage cover categorized in a 1 meter quadrant in each sector. Duff and litter was assessed for fuel load and the number of current year and previous year Monterey pine seedlings were counted in each sector. When looking at the plots today, the differences are hardly visible.

The first three years there was not much different in the mean number of seedlings in each treatment, but in 2003 it was significantly different. New data for 2004 shows more seedlings in the control sector. In 2003 there were significantly more seedlings in the mineral sector.

The study concluded that over the first two years post treatment, regeneration was not significantly different between the treatments. By 2004, more regeneration was found in the sectors that were exposed to bare mineral soil. Survival of the seedlings will be monitored, and the resistance to pitch canker will be determined. Recovery of other vegetation in the treated areas will also be assessed.

### **Impacts of Pitch Canker and Other Biotic Agents on Monterey Pine Forests regenerating Following a Wild Fire** by David Wood

After the 1987 wildfire at Huckleberry Hill on the Monterey peninsula, Huckleberry Hill plots were established in October 1998 of regenerating Monterey and Bishop pines. The plots consist of groups of 30 trees closest to a randomly selected tree along a fire trail or other forest edge. Assessments of pitch canker and tree mortality were made from October 1998 to November 2003. All of the trees were marked with metal tags and GPS locations were obtained for plot centers. From April 2001 onward, the data collection was intensified to include information on pitch moth, tip moth, western gall rust, dwarf mistletoe and other signs of damage.

The study concluded that the incidence of pitch canker is higher in Bishop pines than in Monterey pines. There has been no change in the percent of trees with pitch canker over the last few years. Tree mortality is higher in Monterey pine than in Bishop pine. This mortality is mostly due to self-thinning, the trees are generally less dense in Bishop pine plots. The interaction among the agents will be analyzed.

## **Regeneration of Old Growth Monterey Pine Forests in California Through Gap Creation** by David Wood

The object of this study was to demonstrate the role of canopy openings in the regeneration of Monterey pine in native, senescent Monterey pine forests in central coastal California.

Plots were set up in Pescadero (5 plots), Sunridge x Ronda (3 plots), Lopez x Forest (6 plots) and Fire Road #4 and #6 (3 plots). Each plot contained 3 treatments: 1) a single tree (control); 2) a single tree (treated); and 3) 3 adjacent trees (treated). The treatments consisted of girdling the main stem with a chain saw. The trees still looked healthy, but it was known that they would die. They created one girdling band that was the width of the saw. If the tree does not die, they will be re-girdled. Dr. Volger asked if they saw any evidence of increased cone production, as that is a standard technique. Dr. Wood stated that they just wanted them to die like it was natural, as if a bark beetle killed the tree. He stated that it was important for the study that the tree die.

There will be vegetation assessments of these areas. For the Monterey pine regeneration, their responses will be recorded within a 5 mile radius of each tree over 5 years every 6 months. The seedlings will be categorized as follows:

- Current year seedlings
- 2-year old seedlings
- Greater than 2-year old seedlings, but less than 10 cm in height
- Greater than 2-year old seedlings between 10 and 30 cm in height.

They have removed all the seedlings from these areas so that everything that comes up is new.

The other plant species within a 5 mile radius of each tree will be assessed, recorded and identified to species. The percent cover will be categorized.

## **Assessment of Monterey Pine Regeneration in Naturally Occurring Gaps** by David Wood

In order to assess Monterey pine regeneration in naturally occurring gaps, they randomly selected transects as starting points from a GIS layer in large forested areas. Each transect contains 6 plots at 0, 20, 40, 60, 80, and 100 meters. Each distance constitutes a plot center.

The subject tree is determined as the closest tree less than 2 meters from the plot center. The height, diameter at base and one year growth is measured. A densitometer reading is taken directly above the tree. The tree is cored or cut to determine the age.

Within 1 meter from the plot center the duff depth is measured and the seedlings are classified. The surrounding forest structure at 20, 40, 60 and 80 meters is determined and the age, DBH and height of the closest dominant Monterey pine is measured. These same measurements are taken if a second stratum of Monterey pine is present.

Jack Marshall asked if the tree diameter was taken at the base. Dr. Wood stated that it is taken at the base for the small ones, but the bigger trees are not measured at the base. Mr. Hawley asked if they go to the pith in taking the core. Dr. Wood said that they did, because it helps them know what the age distributions are in this forest and where regeneration shows up. This should correlate to whatever favors natural regeneration. Dr. Volger asked the age of the oldest known Monterey pine. Dr. Woods thought it was 120 years, but said he didn't know the ages of those trees. Trees of 150 to 200 years were

discussed, and Mr. Hawley noted that the core samples from the islands were still sitting in Berkeley, but that the core sampler wasn't long enough when the core was taken.

Dr. Wood added that he thought that there are some pockets of old growth forests that have not been affected by human activity. Where there is only mineralized soil with good weather conditions there is good regeneration in the forest area after the machines have gone through and the canopy has been opened up. He added that as the light is increased and the soil is mineralized, it tends to enhance regeneration. Dr. Woods noted that there was very little evidence of chain saw activity in the forest on the Monterey Peninsula. Chairman Staub stated that there hasn't been much activity since the late 1940's, but that a couple of million board feet were taken out in the 1940's. Chairman Staub noted that in the dense areas there was a much lower regenerate rate. He asked if they could characterize regeneration within the understory types. Dr. Wood stated that their plan is that they will be able to characterize the age, size, species distribution and the understory vegetation.

### **Studies of Phoresy of *Fusarium circinatum* on Potential Beetle Vectors** by Gabriela Ritók-Owens

The objective of the study is to refine integrated pest management strategies for pitch canker by determining the relative importance of each of the known insects associated with *Fusarium circinatum* and by determining the risk that wood infesting insects may carry the pathogen.

The study sites were in Monterey County on the Monterey Peninsula, in Santa Cruz County in Año Nuevo, and in San Luis Obispo County in Cambria. Beetles were collected in 2003 from Monterey County and Santa Cruz County. San Luis Obispo was added in 2004. The baits used were alpha-pinene and ethanol for *I. mexicanus* and other wood boring beetles and ipsdienol and cis-berbenol for *I. plastographus*. The collections were done monthly from April through November for *I. mexicanus* and other wood boring beetles and were done monthly from July through November for *I. plastographus*. The most common beetles were examined for the presence of *Fusarium circinatum*.

The conclusion of the study was that *Fusarium circinatum* is phoretic on *Asemum nitidum* (Cerambycidae) and *Gnathotrichus retusus* (Scolytidae). Both species colonize the sapwood of Monterey pine, therefore logs pose a risk of establishing new infestations. They found the first estimates of colony forming units for an insect-vectored pathogen. *I. mexicanus* carried a large number of propagules. How this affects vector potential still needs to be determined. *F. circinatum* was found on 2 out of 3 deep-wood inhabiting insect taxa: Cerambycidae, Scolytidae, and Siricidae (wood wasps).

Future research should be done to determine the rate of inoculation of Monterey pines in nature with naturally occurring and elevated propagule loads on *Ips* species and *Pityohthorus* species. It would also be helpful to rear deepwood beetles from infected host material.

Chairman Staub asked what was the smallest size of material that *I. mexicanus* could colonize, for example could they colonize a branch or a stem. Ms Ritók-Owens stated that it was hard to say because they hung traps on trees that were in the stand, but not necessarily on the Monterey pine trees themselves. Chairman Staub stated he was trying to figure out if branch tip infections were *Ips* or *Pityohthorus*.

Dr. Wood stated that *Ips mexicanus* seem to tunnel out to the shoot. He stated that they have seen *Ips mexicanus* tunneling up into the area where the cones are pressed into the branch. So that is a potential site. In Bishop pines we see infections in cone whirl. Dr. Gordon asked if these were galleries or re-emerging adults. Dr. Wood stated that he didn't know if they produce progeny in there. Ms Gillette posited that it was because the trees are sending nitrogen to those areas of the trees. Dr. Volger stated that *Ips* has a wide host range within pinus.

Chairman Staub asked what would be the method of spread if it is a wood bearing insect. It was explained that they thought that the trees are dead, but when they leave the trees, they may be leaving the propagules and spores. Chairman Staub thought it sounded like a remote possibility. Dr. Wood noted that the deep wood insects are the second wave insects that are tunneling into freshly killed trees.

It was determined that the information obtained is sufficient to condemn log movements, even logs that have all the bark removed. It was stated that if it is freshly cut, stripped and moved quickly, there is virtually no chance of deep beetles, but if it is out in the forest for a few years, it is a different story. There is so much that changes the environment in that situation, so log history is the key. However, at least the connection between the pathogen and the deep wood insect has been determined.

Mr. Hawley asked if the beetles would go into a tree that was still alive where a major limb was broken off. Dr. Wood stated that the tree usually has to be dead or a large part of it has to be dead in order for them to colonize. But it could be a standing tree or a log.

### **Conophthorus spp. Cone Beetles and Pityophthorus spp. Twig Beetles** by Nancy Gillette of USDA

Ms Gillette stated that the idea of this study is to use beetles as spore sentinels. Candidates for pitch canker vectors to Douglas fir are:

- *P. pseudotsugae*
- *p. orarius*
- *p. setosus*
- *p. carmeli*
- *p. netidulus*
- *p. californicus*
- and other shoot, cone and seed insects (e.g. *Leptoglossus occidentalis*)

They are looking at a broader range of semiochemicals for trapping the beetles. It is unknown terrain at this point. Ms Gillette showed photographs of beetles attacking cones. The pheromone is released in the frass. The female constructs a gallery, attracts a male. They mate in the gallery, construct an egg gallery and lay eggs. This is adaptive for insects because that is where the nitrogen is and the insects feed on that part of the plant. When a tree is bearing a lot of cones, it mobilizes a lot of nitrogen and carbohydrates and the insect burrows into the tree.

Cone beetles and host species were tested;

- *C. ponderosa* on *P. ponderosa*
- *C. ponderosae* on *P. monticola*
- *C. conicolens* on *P. pseudostrobus*
- *C. teocotum* on *P. teocote*
- *C. coniperda* on *P. strobes*
- *C. resinosae* on *P. resinosa*
- *C. michoacanae* on *P. michoacana*

A lot of the work was done in Mexico. *Cono* is closely related to *Pity* so they use the same chemicals when they are collected. When *Cono* was collected, they got a lot of *Pity* in the traps that have not been analyzed to determine the active pheromones for the active *Pity* species.

They want to determine whether they can use trapped beetles as sentinels for pitch canker, particularly in Badger Hill, using the beetles to determine it, rather than sending people out there to take samples.

The behaviorally active chemicals for cone beetles are:

- Pityol – female beetles
- Conophthorin (male/female beetles)
- Alpha-pinene (pines)
- Verbenone (female beetles)
- 4-allylanisole (pines, many angiosperms)
- 2-Hexenol (“green leaf volatile”)

The behaviorally active semiochemicals for twig beetles are:

- Pityol (attracts)
- A-pinene (synergist)
- Conophthorin (attracts)
- 2-hexenol (synergist)
- Verbenone (synergist/repellent)
- 4-allylanisole (synergist/repellent)

Ms Gillette stated that they typically don't use lingren traps, but use smaller traps. Because the traps are small and portable, they can do bigger studies with 100 traps at each site. They attempt to get the traps in the top third of the tree. Most of the stands are more open with branches close to the ground, so they can throw a rope over the branch. She stated that they want to do parallel studies, with one on the coast where it is known that pitch canker occurs. They would like to culture half the insects trapped at Badger Hill and save the other half for identification. This way the vectors to Douglas fir can be identified and it can be determined how effectively the trapped beetles can be used as evidence for pitch canker.

Mr. Hawley asked how many eggs can be produced each year. Ms Gillette stated that approximately 50 eggs can be produced but they are limited by the size of the cone. Mr. Hawley asked if multiple insects would attack one cone. Ms Gillette answered that once the female is mated, she produces a pheromone that gives notice to the other cone beetles that the resource is taken; so there is rarely more than one couple per cone.

Chairman Staub asked if the tip die-back that is being observed in Monterey pines was *Pity*. Dr. Wood stated that Andrew Storer just did a paper on that subject. He stated that they put spore suspension on beetles and put them in cages on the tree. Approximately 30% of the trees had transmission. This provides evidence that the beetle is doing something to wound the tree, but they are not sure exactly what is occurring. Dr. Wood described the cone beetle as a beautiful vector which goes right into the developing shoot and in this case the beetle enters the cone entirely.

Jack Marshall asked if this was the same insect as that for flag tips. He asked if when flagging is observed only at the top of 60 – 70 foot trees, could it be *Pity*? Dr. Wood stated that this is not a sign of *Pity*. He stated that dead tips in the upper third of the crown is caused by pitch canker and pit moth. *Pity* colonizes branches in the lower crown.

Chairman Staub asked if Dr. Wood would conclude that the vector is cone beetles. Dr. Wood stated that attracting the insects to the trees increased pitch canker. *Cono* is in the one- or two-year old cones. Ms Gillette added that there is a reason why the distribution of pitch canker is similar to the distribution of

cone on the tree. The cone beetles attack only living cones. Dr. Gordon added that one interesting consequence is that it will most likely overcome some of the environmental limitations; because, since they go in that deeply, the environmental limitations can't affect it. Mr. Hawley asked if pitch canker could get into the seed through the cone. Dr. Gordon stated that it could not get in directly because the cone is not going to make it. Internally infected seed is found where the branch is infected further back. He added that at this point they are not certain how the fungus gets in there.

### **The Impacts of Pitch Canker on Susceptible Pines in California** by Thomas Gordon

The study found that the effect of repeated inoculations on susceptibility over time was that the inoculated trees consistently showed shorter lesions over time. They were interested to discover if this was an artifact of the experiment.

They observed in 1999 that many of the 1996 trees in the severely diseased category were not diseased anymore. The old infections were not seen anymore because they have broken off. The symptoms are no longer visible because of new branch growth. The question was why there are no new infections. They concluded that the trees were now resistant. This conclusion was tested by reinoculating the trees with pitch canker with the standard test and harvesting the branches. The results were dramatic. It showed that virtually all of the trees in remission were now in the resistant category. This showed up in a variety of locations. Virtually all of the trees are now free of disease. They next wanted to determine if trees in stands with a history of pitch canker are more resistant than trees in stands more recently exposed.

In the experiment they created 16 plots. Eight of the plots were in "old sites" and 8 were in "new sites". There were 20 trees per plot. Three branches per tree were inoculated. When measuring the mean lesion lengths after the first round of inoculations they found that the lesions in the old sites were on average about half the length of the lesions on trees in the new sites. Additionally, the mean number of killed branches in the old sites was 1.9 per plot compared to 6.4 per plot in the new sites.

A second round of inoculations was completed in 2004. In the 16 plots, they re-inoculated 20 trees and inoculated 20 more trees for the first time. They inoculated 3 branches per tree for a total of 1920 lesions.

Dr. Gordon stated that this is where the research now stands. It is important in the context of pitch canker. He stated that they don't know how long the resistance lasts and why some trees manifest resistance while others don't. They also don't know how it plays out in other pine species. This is probably very important from an evolutionary perspective. He added that if it is a genetic contest, the trees will lose. However, now it is felt that there are mechanisms that will allow the trees to adapt.

Dr. Gordon acknowledged all the landowners who were willing to work with them on this program and the people who worked on the project. He also acknowledged funding from USDA Exotic Pests & Diseases Program.

Chairman Staub congratulated Dr. Gordon on this interesting information. He asked if there was any speculation as to what biological pathway had been activated in the trees. Dr. Gordon stated that if they extrapolate from other models, it seems to be certain signaling compounds that regulate all the defense genes. He stated that it could well be something like that, or at least that could be part of the picture. It also could be a change in the resin composition. He added that it is hard to believe that it is specific to pitch canker, but it probably is rather a general elevation of the defense of the plants.

Mr. Hawley asked about whether *Fusarium circinatum* might be targeted or not. He stated that he didn't observe the phenomenon of remission occurring with mistletoe or western gall rust. Dr. Gordon stated

that to the casual observer they might not notice it unless they monitored the disease status of individual trees. He added that he didn't have an answer as to why it responded this way to this pathogen. He added that this is an introduced pathogen, and you could question if it is more likely in an introduced pathogen as opposed to a co-evolved pathogen. He added that he could see it either way.

Don Owen stated that it has been reported that in the southeast they never see pitch canker outbreaks in the same location. He speculated that this could show that there might be induced resistance. Dr. Owen added that with pitch canker they are inoculating the tree with all these little inoculations all over the tree. Usually it occurs over the years without the trees dying; it takes years for the trees to die. Dr. Wood asked that if some of the trees are so affected by pitch canker in 1992, why weren't they killed by bark beetles. Dr. Gordon acknowledged that it sounded like something that they should work on.

### **BADGER HILL UPDATE**

Dr. Det Volger reminded the Task Force that he had reported an infestation of pitch canker on Badger Hill for the first time a year ago. He stated that he wanted to go back and explain some of the history of that discovery.

Dr. Volger said that the Forest Service has had a major genetic program on Badger Hill for the last 30 to 40 years. One of the main goals of the genetic resources program over large land acres is genetic conservation. The largest and most obvious resources are the trees. The purpose of the program is to do in situ conservation, placing genetic material into repositories. One of the major repositories is at Badger Hill. Forest Hill and Badger Hill have a rich on-site storage of genetic diversity in the form of the trees. The trees are set up in certain designs so that trees of similar preferences will tend to pollinate each other. Seeds can be collected from these trees, as can scion wood, and new trees can be produced. The Forest Service also works with private industry because their holdings are so widely scattered, so they can share genetic material.

Cuttings and scion wood are taken from Badger Hill and sent to Oregon and then on to New Zealand. These cuttings and scion wood are grafted onto New Zealand trees, and seeds can then be produced much more quickly. In New Zealand, the trees were grafted in quarantine and kept there for nine months. At the end of this process, around August of 2003, it was found that one tree had pitch canker. That is one tree out of thousands of grafts. The ultimate source of the material from which the pitch canker originated was California. No one knows exactly what was done with the material from the time it left California and arrived in New Zealand.

Pitch canker was found on Badger Hill in November of 2003. Dr. Thomas Gordon and others collected samples from the tree upon which the pitch canker was found and other trees in the area. The branch of a Douglas fir root stock had died back about 8 inches. They did not know if it was diseased, but they found that it was infested with the pitch canker fungus. It is important to note that it was infested, as everything is infested and everything is contaminated. However, if it was diseased that would indicate some expression of physiological change that has some consequence. Pitch canker is the disease; pitch canker fungus is a fungus. It can be topical without causing the disease.

Pitch canker was found in Badger Hill because the material that is deposited there is extremely economically and socially valuable. It has been looked at carefully. The consequences of pitch canker in Badger Hill are that it would wash out something that is totally irreplaceable. Potentially 35 years of effort would be cut down in one day. Dr. Volger stated that they are trying to find that out – is the problem sufficient to destroy 30 years of conservation effort.

Dr. Volger passed around a handout that demonstrated that the tests done every two months from November 2003 through March 2005 produced negative results on every tree sampled at Badger Hill. He stated that they are just completing the first replication of the find of pitch canker fungus in Badger Hill.

Dr. Volger said that when the initial examination was done on Badger Hill, Dr. Gordon walked every single road. He stated that originally there was just the one positive in the material from New Zealand and one more positive in California. On February 19, 2004, another collection confirmed four additional positives for pitch canker fungus at Badger Hill. They determined that one was symptomatic. They considered a tree symptomatic when it had any sort of lesion which led to a deformity of the branch, even color change. The tree was symptomatic, but it may be that the pitch canker fungus was not the cause of the symptom. Most of the collections that were made were probably not diseased, but were just damaged in some other way. Dr. Volger noted that the symptom was always at the terminal end of the shoot and that some of the time it was way out on the branch. The remainder of the early symptomatic branches proved to be negative. In February 2004 there were four trees and the original tree that were possibly infested. At that time the Task Force recommended that the original tree be cut down. It was uncertain if the other four trees should be cut. In early March, two trees were cut with cameras running.

Since that time, bimonthly assessments have been done for anything symptomatic plus 60 asymptomatic branches. Once identified, they were sampled repeatedly. The trees have been retested and the trees on Forest Hill and Happy Camp have been tested and there is nothing there.

Richard Hawley asked if the source of the sporulation has been found. Dr. Volger stated that they don't know the source of the fungus. He noted that the Christmas tree industry in that area is getting larger and that they grow Douglas fir. He stated that there have been no outbreaks in the area that were important enough for the Christmas trees growers to be concerned. The state pathologist and the Ag extension staff are so over-extended, however, that there has been no active survey of Christmas tree farms. Also, there is a timeshare campground in the area. It is likely that firewood is being brought to the area by people coming to stay there. Another source could be insects or spores which are being brought up from the coast. There is an ozone air pollution pipeline from the Bay area past Sacramento over the Tahoe basin. They are a major receptor of pollution from the coast. It is major east-northeast flow across Placerville.

Dr. Volger stated that Dennis Chamberlain has helped with this project a lot by learning everything he could at the Davis lab. In April 2004, a lab was established at Placerville. The Pacific Southwest station had been without any personnel for over ten years, but now a pathology lab could be built. Also, it was important to have a lab in El Dorado County so that the material did not have to be taken out of the county to Davis for testing. Now all the raw material is tested in Placerville and then the Petri dishes are wrapped and sent to Davis.

Dr. Volger said that the isolate found in New Zealand was VCG2. The five found in California were VCG1, which tentatively looks like a problem. This is different than what was earlier reported. However, VCG1 and VCG2 are almost genetically identical.

Additionally, Dr. Volger stated that they haven't determined that the symptoms had anything to do with the topical find of the pitch canker fungus. They cannot say for sure. He noted that in October they were collecting every month at Badger Hill, but they will now be making two collections per month. In October they collected something that looked like the fungus, but learned yesterday from the Davis lab that the isolate was determined not to be pitch canker fungus. The lab will go on to determine what it was that they found.

Dr. Volger noted that the only pitching is by lepty on cones. They have been collecting cones, but pitch canker fungus has not been seen on them. Last year pollen was collected from 24 trees which will be tested. They hope to continue to collect through the temperate time of the spring, hopefully through some time in April.

Dr. Volger stated that this site, where the Douglas fir is planted, also contains Monterey knobcone, which was supposedly resistant to a lot of things including ozone. It has been decided to plant Monterey knobcone as plant sentinels and they will periodically go out to check them. There are also plans to investigate other areas in El Dorado County.

Dr. Volger concluded by stating that there is no conclusive evidence that the pitch canker disease is in Badger Hill. There is no conclusive evidence that the original infestation was in or around Badger Hill. Also, there is no conclusive evidence that the pitch canker found in New Zealand was from California. He stated that at the end of this process, if no more pitch canker is found, they will meet with Forest Service representatives, industry representatives and others in order to decide how to proceed.

### **MAPPING PROJECT**

Dr. Susan Frankel reported on the project that was discussed at the last meeting regarding a pitch canker GIS. She reported that since September, things have changed. A proposal was submitted for a GIS base for monitoring, but it wasn't funded. Eventually someone agreed to take on the project, but they will be doing the work in-house, so it will be a national database for pitch canker and will include the southeast. Dr. Gordon will work with the database developers. He will make a template of what to put in the database and perhaps at the next meeting someone will be able to come to the Task Force and make a presentation of a prototype of the project. Only pitch canker finds confirmed by the laboratory will be included. If anyone other than Dr. Gordon's lab has some of the data to put in the database, please let them know. They will wait to decide on the map protocol. They are trying to report every confirmed location – tree by tree – as well as study sites. The location of confirmed disease sites or confirmed sites where the pathogen is found will be shown by substrates.

Chairman Staub asked if this means that the project will be funded. Dr. Frankel stated that the Forest Service said that they have a programmer who will work on it for us.

Dr. Frankel told the Task Force that, starting March 6<sup>th</sup> she will be working at the Pacific Southwest Research Station, while she will still be trying to do her other job as well.

### **IMPACT PROJECT UPDATE**

David Spencer was introduced as a guest at the Task Force meeting from the national federal research body in Australia, CSIRO – Forestry and Forest Product Division. He has been a tree breeder for 40 years. He came to the states for this project. He reported that they have just begun a trial at Swanton Pacific with a tree planting that was designed for maximum level of sensitivity. He is sure there will be great information coming out of the study. Mr. Spence came over from Australia to help with the design of the project and to do any background that had to be done. He noted that there were six people today at the Task Force meeting out of the 25 people who planted the trees. Right now there is no one watching over the seedlings, just the general ranch staff. But he added that the seedlings are all fenced off for cattle exclusion.

## **CDF NURSERY**

Steve Jones stated that he is responsible for the CDF nursery program. In the 1990's, CDF stopped production of Monterey pine seedlings and they haven't collected any seeds since then. He added that there are no government seedlings or seeds available. He was asked about collecting seeds but not growing them, just increasing the seed bank. He stated that they still have seedlings storage from pre-pitch canker days. He added that they didn't want to create a possible bridge to the Sierras.

## **ACTION PLAN**

Copies of the Pitch Canker Task Force Action Plan were distributed to the Task Force members.

The next meeting was scheduled for September 22, 2005 at Swanton Pacific Ranch.